Exhibit A

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Coordinate dual-gene transgenesis by lentiviral vectors carrying synthetic bidirectional promoters

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Transferring multiple genes into the same cell allows for the combination of genetic correction, marking, selection and conditional elimination of transduced cells or the reconstitution of multisubunit components and synergistic pathways. However, this cannot be reliably accomplished by current gene transfer technologies. Based on the finding that some cellular promoters intrinsically promote divergent transcription, we have developed synthetic bidirectional promoters that mediate coordinate transcription of two mRNAs in a ubiquitous or a tissue-specific manner. Lentiviral vectors incorporating the new promoters enabled efficient dual gene transfer in several tissues *in vivo* after direct delivery or transgenesis, and in a human gene therapy model. Because divergent gene pairs, likely transcribed from shared promoters, are common in the genome, the synthetic promoters that we developed may mimic a well-represented feature of transcription. Vectors incorporating these promoters should increase the power of gene function studies and expand the reach and safety of gene therapy.

Expression of multiple transgenes within the same target cells is required for several gene transfer and therapy applications[1]. Gene-function studies are best performed by expressing cDNAs together with a marker gene; by this approach, genetically modified cells can be identified *in vitro* and *in vivo*. Similarly, gene therapy applications can be improved by purification of gene-corrected cells before *in vivo* administration, taking advantage of coordinate expression of selectable markers. Genetically modified cells can be amplified by introducing growth promoting or drug resistance genes together with the therapeutic gene. Conversely, genetically modified cells expressing conditionally cytotoxic genes, together with the therapeutic gene, can be eliminated *in vivo* if adverse events occur.

Coordinate expression of more than one transgene is essential when the activity to be reconstituted by gene transfer depends on multiple subunits encoded by different genes, or requires the synergism of separate molecules. In spite of such well-recognized needs, reaching coordinate, high-level expression of multiple transgenes in the majority of target cells has been a significant challenge for gene transfer technology. Two different transgenes can be expressed by two separate vectors; yet, only a fraction of target cells is transduced by both vectors and a heterogeneous population of cells is obtained that expresses either one or two genes in different ratios. Alternatively, different promoters within the same vector have expressed two or more transgenes; yet, different tissue specificity and mutual interference between promoters often prevented efficient coexpression in the same target cells[2,3].

Polyproteins that self-process cotranslationally into separate components have been generated using self-cleaving 2A-like peptides of the Foot and Mouth Disease Virus (FMDV) and other picoRNA-viruses[4,5]; however, application of this technology has been limited until recently because it requires molecular engineering of both transgenes and introduces sequence changes that may affect the activity, stability and immunogenicity of their protein products. The most common approach to multiple gene transfer has relied on using internal ribosome entry sites (IRESs)[6]. However, several reports have noted important limitations of IRES-based dual gene transfer vectors[6–10], similar to those we describe here for IRES-based self-inactivating (SIN) lentiviral vectors (LVs).

To coordinately express two transgenes from a single gene transfer vector and overcome the limitations of the approaches described above, we explored a new promoter design. We joined a minimal core promoter upstream, and in opposite orientation, to an efficient promoter. The rationale of this design was that upstream elements in the efficient promoter, when closely flanked by core promoters on both sides, may drive transcriptional activity in both directions. If such bidirectional activation occurred, expression of both transcripts would be coordinately regulated.

We first tested two ubiquitously expressed promoters, previously shown to drive robust and efficient transgene expression in LVs: a 516-bp fragment from the human phosphoglycerate kinase promoter (PGK)[11] and a 1,226-bp fragment from the human ubiquitin C promoter (UBI C)[12]. We joined them to a minimal core promoter derived from the cytomegalovirus (minCMV) that was previously developed to couple initiation of eukaryotic transcription to tetracycline-dependent operators[13]. We flanked the bidirectional promoter with two expression cassettes optimized for LV-mediated gene delivery (**Fig. 1a**). The upstream cassette—in antisense orientation relative to the vector LTR—included the constitutive transport element (CTE) of the Mason-Pfizer virus[14], and a polyadenylation site from the Simian Virus 40 (SV40). The downstream cassette included

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the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) and the SIN HIV-1 LTR polyadenylation site[11].

We cloned the luciferase reporter and the cell-associated green fluorescent protein (GFP) marker genes into the bidirectional expression cassette and generated vesicular stomatitis virus (VSV)-pseudotyped stocks of bidirectional and control monocistronic LVs. LVs carrying bidirectional expression cassettes were produced to high titer and infectivity, approaching those obtained with standard vectors. We verified correct transfer and normalized transduction of each vector stock by Southern blot analysis and/or real-time PCR of transduced HeLa cells (data not shown). We then compared gene expression in target cells transduced with equal numbers of vector copies. The bidirectional design significantly enhanced transcription from the upstream minimal promoter without affecting downstream expression from the efficient promoter (**Fig. 1b–h**). Luciferase expression from the minCMV promoter, for instance, increased at least one log when fused upstream to the PGK promoter (**Fig. 1b**). Remarkably, the bidirectional PGK promoter allowed us to detect GFP (or a truncated version of the low-affinity NGF receptor, ΔLNGFR, data not shown) to the same frequency and to similar expression levels in cells transduced by the bidirectional vector and expressing the protein from either side of the promoter (**Fig. 1c,d**), as in cells transduced by the control PGK vector (**Fig. 1e**). Using two cell-associated markers, ΔLNGFR and GFP, we showed stable, efficient and coordinate

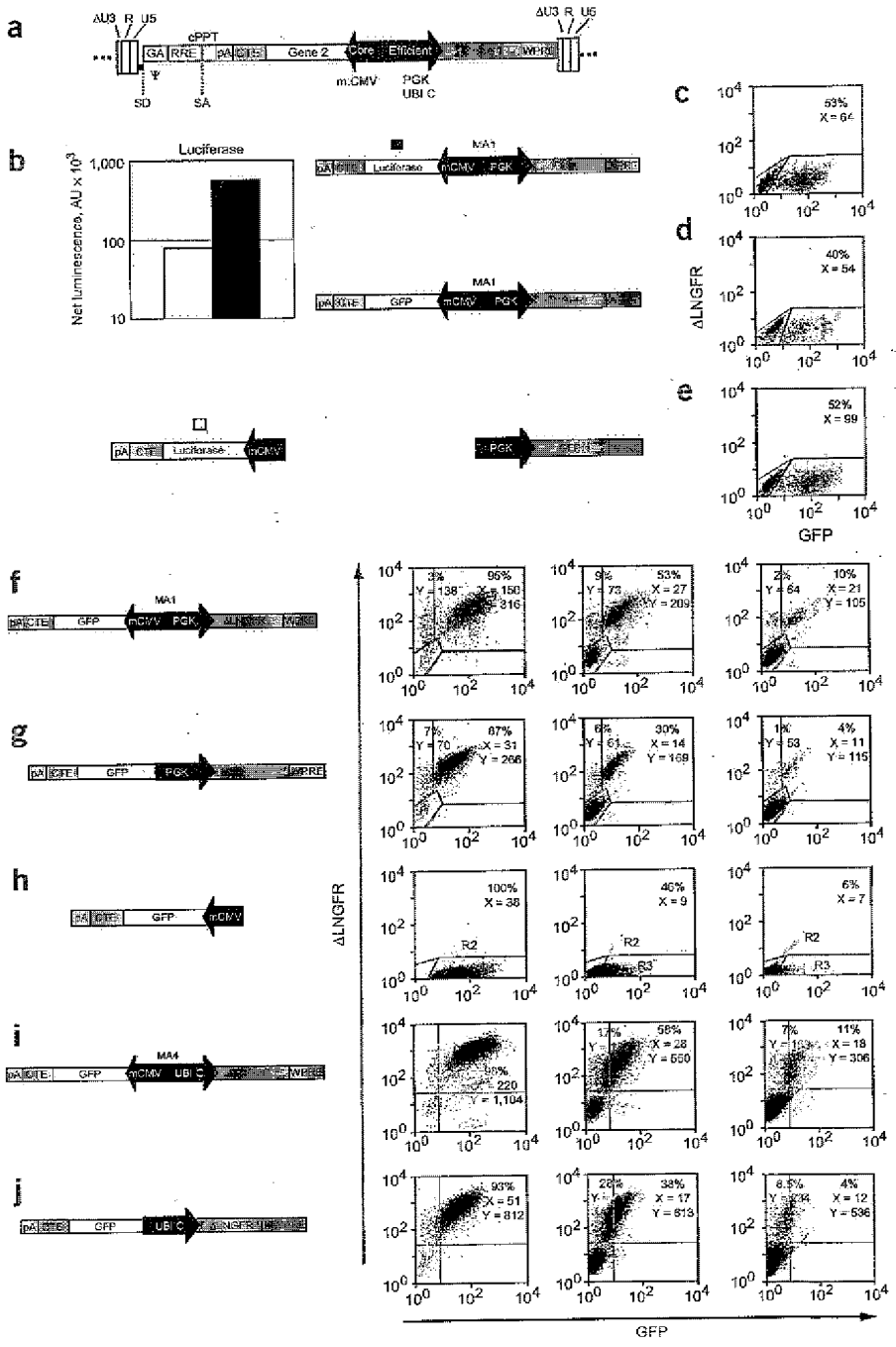

Figure 1 Gene transfer performance of bidirectional lentiviral vectors. (a) Scheme of the proviral vector form. A bidirectional promoter made by minimal core promoter elements from the human cytomegalovirus (mCMV) joined upstream, and in opposite orientation, to an efficient promoter, derived from the human phosphoglycerate kinase (PGK) or polyubiquitin UBI-C gene, was driving divergent transcription of two RNAs. CTE, constitutive transport element from the Mason-Pfizer monkey virus; pA, polyadenylation site A from the Simian Virus 40. ΔU3, R and U5, LTR regions with deletion in U3; SD and SA, splice donor and acceptor site; ψ, encapsidation signal including the 5′ portion of the gag gene (GA); RRE, Rev-response element; cPPT, central polypurine tract; WPRE, woodchuck hepatitis virus post-transcription regulatory element. (**b–e**) Net luciferase activity (**b**) and GFP expression (**c–e**) in HeLa cells transduced 5–7 d before with LVs carrying the indicated bidirectional or control expression cassettes. The frequency and mean fluorescence intensity (MFI) of GFP+ cells at FACS analysis is indicated in the dot plots to the right. Luciferase activity was determined for the two marked vectors (□, ■). X, MFI. (**f–j**) ΔLNGFR and GFP expression in HeLa cells transduced 5–7 d before with serial tenfold dilutions of LVs carrying the indicated expression cassette. The frequency of ΔLNGFR+ (upper left region) and ΔLNGFR/GFP double positive (upper right region) cells, with the respective MFI of ΔLNGFR (Y) and GFP (X), are indicated in the FACS dot plots. Results shown are representative of at least three experiments performed with similar results.

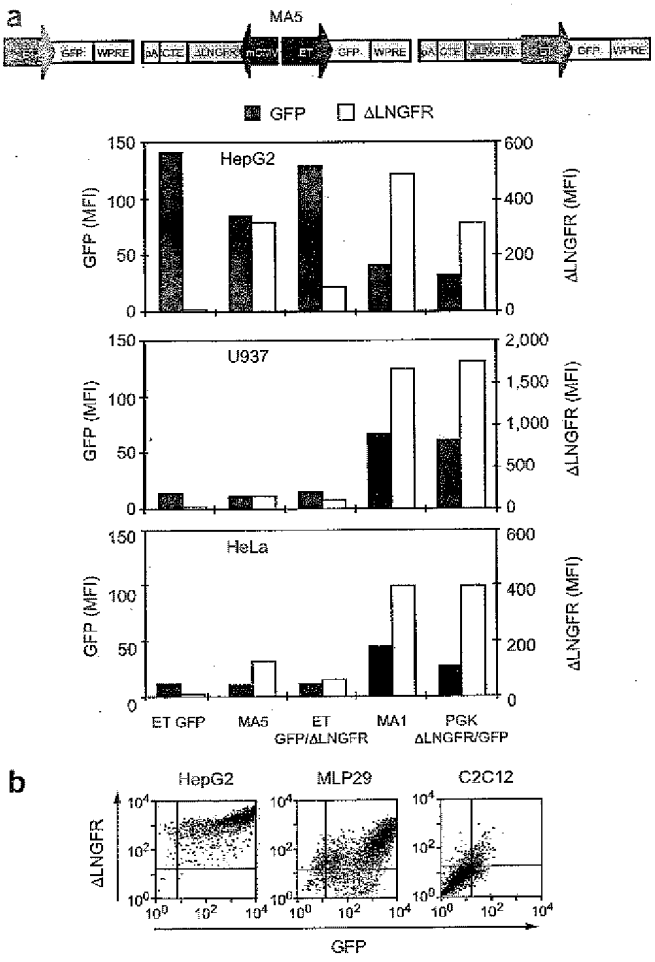

Figure 2 Tissue-specific expression by bidirectional LVs. The indicated cell lines were transduced with matched doses of the indicated monocistronic or bidirectional vectors, either based on the hepatocyte-specific ET promoter (without and with the minCMV promoter, MA5) or on the ubiquitously expressed PGK promoter (without and with the minCMV promoter, MA1; see **Figure 1g** and **f**, respectively, for schematics) and expressing GFP and ΔLNGFR, analyzed by FACS after 5 d. (**a**) Histograms showing the GFP (left *y* axis) and ΔLNGFR (right *y* axis) MFI in cells transduced by a low vector dose, thus representing expression from single vector copies. The mean of two determinations from one of two similar experiments are shown. (**b**) Representative dot plot FACS analysis of GFP and ΔLNGFR expression in target (HepG2 and MLP29) and nontarget (C2C12) cells transduced by a high dose of the hepatocyte-specific MA5 vector.

expression of bidirectional LVs, both at high and low vector copy number (**Fig. 1f**).

At high vector input, we reached high-level expression of both transgenes in virtually every target cell. At low vector input, when most transduced cells carried one proviral copy, we showed transgene coexpression in virtually every labeled cell, indicating the occurrence of divergent transcription from the bidirectional promoter. In both conditions, transgene expression was maintained to similar levels in cells analyzed at early and late time-points post-transduction (data not shown). Transgene-expressing cells tended to distribute along a diagonal line in the two-color fluorescence-activated cell sorting (FACS) plot, indicating that expression of the two transgenes was coordinately regulated. Moreover, by measuring expression in relation to the number of integrated copies, as measured by real-time PCR, and analyzing cells transduced with increasing vector doses, we showed that the vast majority of integrated bidirectional vectors based on the PGK (MA1) or UBI-C (MA4) promoter efficiently expressed both transgenes (**Supplementary Fig. 1** online).

Intriguingly, we observed coordinate bidirectional expression, although to substantially lower efficiency on the upstream side than the downstream side, when we tested the sole PGK promoter in the context of the bidirectional expression cassette that we developed (**Fig. 1g**). Bidirectional expression was detectable at single copy levels and for the vast majority of transduced cells, as assessed by real-time PCR (**Supplementary Fig. 1** online). We reproduced this finding in all cell types tested (see below and data not shown) and after swapping the position of the two transgenes on each side of the PGK promoter (data not shown). These results indicated that transcription-activating elements in the PGK promoter are intrinsically capable of triggering divergent transcription and thus provide the main driving force for dual-gene expression in the new LVs, ensuring coordinate regulation of transcription on both sides of the bidirectional promoter. Apposition of the minCMV core promoter, which had a very low activity *per se* (**Fig. 1b** and **1h**), enhanced upstream transcription from the PGK promoter possibly because of more efficient transcription initiation (compare **Fig. 1f** and **1g**, relative GFP expression level from upstream of the PGK promoter versus upstream of the minCMV-PGK at a single vector copy: 59 ± 4%, mean ± s.d., $n = 5$, $P < 0.01$; this difference became even more apparent with increasing vector input). When we changed the driving promoter in bidirectional vectors from PGK to UBI-C, we reproduced the findings observed with the PGK promoter (**Fig. 1i**). We also revealed an intrinsic bidirectional activity of the UBI-C promoter (**Fig. 1j**) that was enhanced by the upstream addition of the minCMV promoter.

To explore the potential application of the bidirectional promoter design to tissue-specific expression, we replaced the PGK promoter in the above constructs with a hepatocyte-specific enhancer/promoter previously generated by fusing hepatocyte-specific enhancer elements to the murine transthyretin promoter (ET promoter) and tested the performance of the new LVs in a panel of cell lines of different origins transduced by matched vector doses (**Fig. 2**). The synthetic minCMV-ET promoter (MA5) drove efficient and coordinate expression of the upstream (ΔLNGFR) and downstream (GFP) transgenes, with both proteins highly detectable in cells transduced by a single LV copy (**Fig. 2a**). Notably, efficient vector expression was restricted to hepatocyte-derived (human HepG2 and murine MLP29) cells among the cell lines tested (HeLa cells, human monocytic U937 cells, murine C2C12 myoblasts). In nontarget cells, both the original ET and the new bidirectional MA5 promoters drove a low to undetectable level of expression even after high vector input (**Fig. 2b**). Interestingly, as observed for the ubiquitously expressing promoters, the ET promoter displayed an intrinsic bidirectional activity in its elective target cells that was enhanced by the upstream addition of the minCMV promoter.

We then compared the dual gene transfer performance of bidirectional vectors with that observed for bicistronic vectors based either on IRES elements or self-processing peptides. For the former group we selected the encephalomyocarditis virus wild-type IRES (EMCVwt)[15] as the best performing sequence among a panel of viral and eukaryotic IRESs tested (**Supplementary Notes** and **Supplementary Fig. 2** online). For the latter group, we used the FMDV 2A peptide, whose suitability for coordinate gene transfer was recently highlighted[16]. We compared expression of ΔLNGFR and GFP in HeLa cells transduced with escalating doses of all these vector types by FACS, western blot

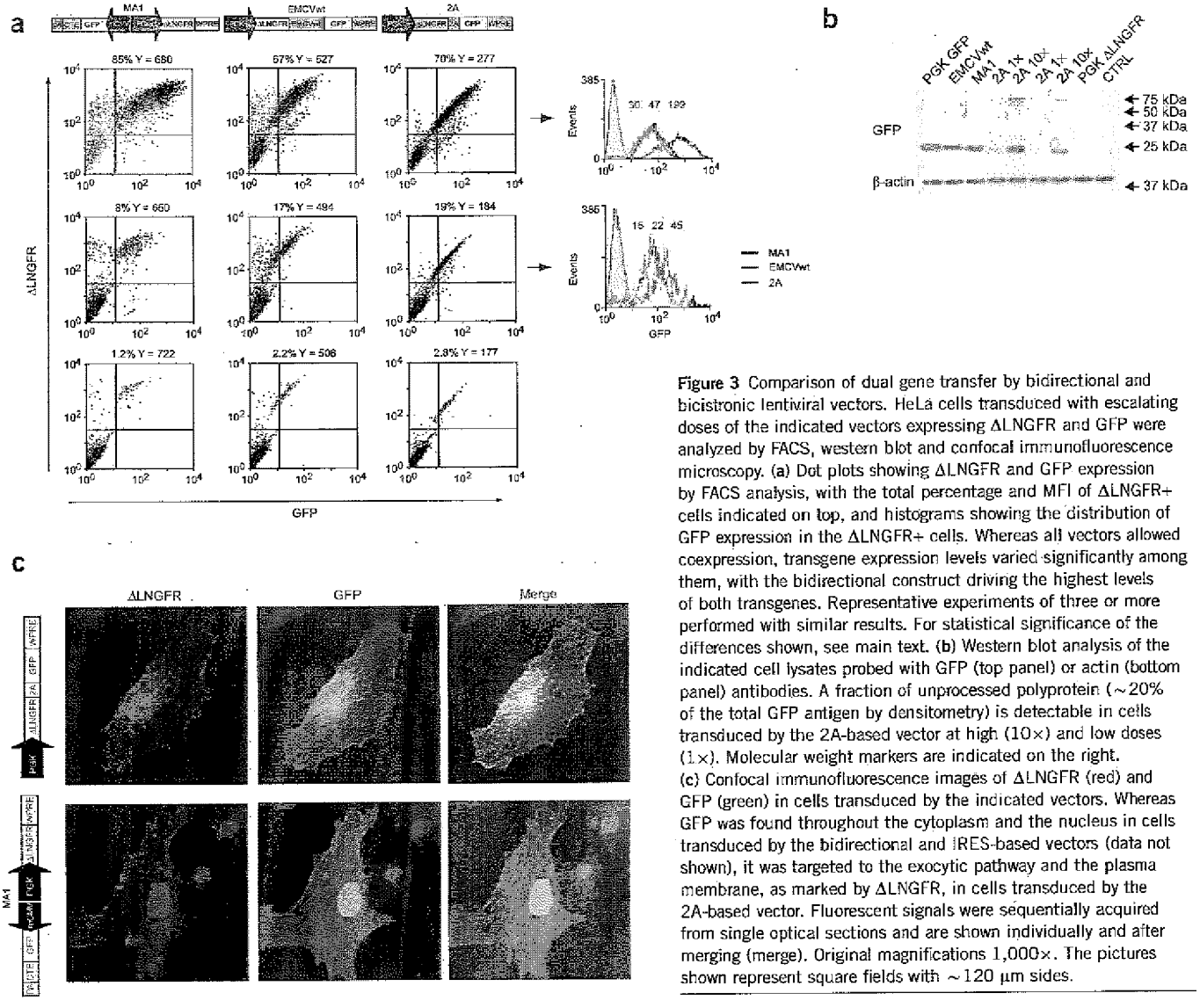

Figure 3 Comparison of dual gene transfer by bidirectional and bicistronic lentiviral vectors. HeLa cells transduced with escalating doses of the indicated vectors expressing ΔLNGFR and GFP were analyzed by FACS, western blot and confocal immunofluorescence microscopy. (**a**) Dot plots showing ΔLNGFR and GFP expression by FACS analysis, with the total percentage and MFI of ΔLNGFR+ cells indicated on top, and histograms showing the distribution of GFP expression in the ΔLNGFR+ cells. Whereas all vectors allowed coexpression, transgene expression levels varied significantly among them, with the bidirectional construct driving the highest levels of both transgenes. Representative experiments of three or more performed with similar results. For statistical significance of the differences shown, see main text. (**b**) Western blot analysis of the indicated cell lysates probed with GFP (top panel) or actin (bottom panel) antibodies. A fraction of unprocessed polyprotein (~20% of the total GFP antigen by densitometry) is detectable in cells transduced by the 2A-based vector at high (10×) and low doses (1×). Molecular weight markers are indicated on the right. (**c**) Confocal immunofluorescence images of ΔLNGFR (red) and GFP (green) in cells transduced by the indicated vectors. Whereas GFP was found throughout the cytoplasm and the nucleus in cells transduced by the bidirectional and IRES-based vectors (data not shown), it was targeted to the exocytic pathway and the plasma membrane, as marked by ΔLNGFR, in cells transduced by the 2A-based vector. Fluorescent signals were sequentially acquired from single optical sections and are shown individually and after merging (merge). Original magnifications 1,000×. The pictures shown represent square fields with ~120 μm sides.

analysis and confocal immunofluorescence microscopy (**Fig. 3**). Whereas all vectors enabled dual gene transfer and expression, we observed significant differences in the expression levels reached by the three types of LV. These differences were already evident when comparing cells transduced to low frequencies (between 1 and 20%), in which the vast majority of transduced cells contain only one vector copy (see also **Supplementary Fig. 1** online).

Expression of ΔLNGFR, the transgene immediately downstream to the PGK promoter in all constructs, was significantly lower for the 2A-based vector and, although to a lesser extent, for the IRES-based vector, as compared to the bidirectional vector, which expressed the protein to levels similar to those reached by monocistronic PGK vectors (average expression level 29 ± 3%, $n = 4$, $P < 0.01$; and 64 ± 17%, $n = 5$, $P < 0.01$, for the 2A-based and IRES-based LVs, respectively, versus bidirectional LVs). Of note, an even more significant decrease in the upstream gene expression level as compared to bidirectional or monocistronic vectors was observed from IRES-based vectors when they were used to express transgenes other than ΔLNGFR or to transduce primary cell lines (**Supplementary Notes** and **Supplementary Fig. 2** online; see also the work of other groups[8–10]). In addition, expression of GFP from the downstream position in both bicistronic vectors was significantly lower than that observed from the upstream position in the bidirectional vector (average expression level 50 ± 5%, $n = 3$, $P < 0.01$; and 40 ± 8%, $n = 4$, $P < 0.01$, for the 2A-based and IRES-based LV, respectively, versus bidirectional LV). As a consequence, in cultures transduced by bicistronic vectors, only the cells expressing ΔLNGFR to high levels reached detectable GFP coexpression.

The described differences in expression were even more apparent with increased vector input (**Fig. 3a**). When we monitored the self-processing of the 2A polyprotein by western blot analysis using GFP antibodies, we found that ~20% of the antigen in transduced cell lysates displayed a molecular weight consistent with that of an unprocessed ΔLNGFR-2A-GFP protein (**Fig. 3b**). Surprisingly, expressing GFP together with the membrane-bound ΔLNGFR in the context of a 2A peptide-based polyprotein appeared to retarget most GFP molecules, both unprocessed and processed, to the exocytic pathway (**Fig. 3c**).

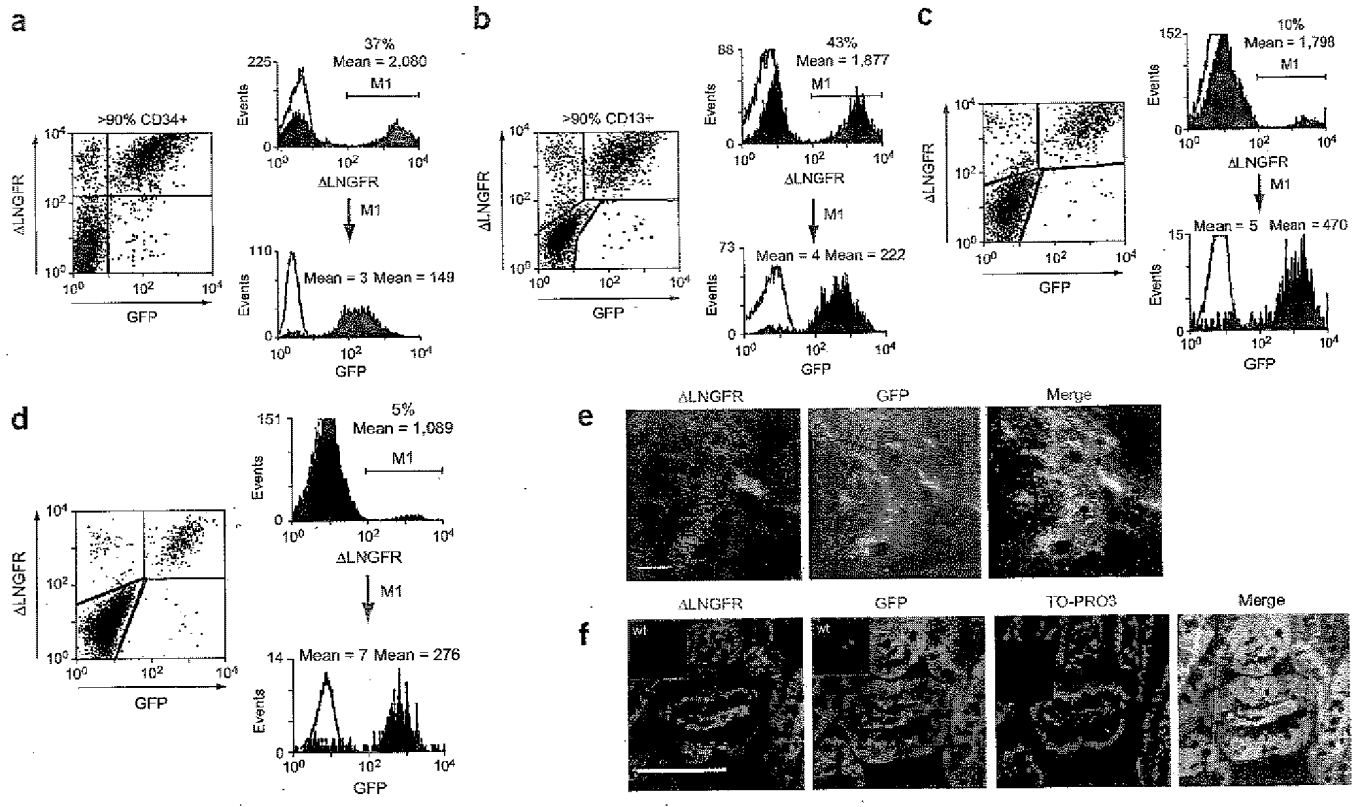

Figure 4 *Ex vivo* and *in vivo* dual-gene transfer and therapy by bidirectional vectors. (**a**,**b**) Human cord blood CD34+ progenitors were transduced by the ΔLNGFR/GFP MA1 vector in the presence of early acting cytokines as described[17], and analyzed either after 7 d of culture in the same medium (**a**), and after additional 10 d in medium promoting myeloid differentiation (**b**). For **a** and **b**, a dot plot showing ΔLNGFR and GFP expression by FACS analysis is shown, together with histograms showing the distribution of ΔLNGFR expression in all viable cells analyzed (top), and of GFP expression in the gated (M1) ΔLNGFR+ cells (bottom). The percentage of immature progenitors expressing CD34, and of differentiating cells expressing the CD13 myeloid marker at the time of analysis is indicated. (**c**,**d**) Human peripheral blood lymphocytes (PBL) were transduced either after 2-d activation with anti-CD3 and anti-CD28 antibodies (**c**), or after 4-d treatment with IL-7, as described[18], (**d**), and analyzed for ΔLNGFR and GFP expression as described above. Experiments shown are representative of three performed with similar results. Cells transduced to low vector copy numbers are shown in **c** and **d** for more stringent performance analysis. (**e**) High-titer of ΔLNGFR/GFP MA1 LV was stereotactically injected into the striata of adult mice. Cryostatic brain sections were obtained two months after injection and analyzed by immunofluorescence and confocal microscopy. Representative pictures of the injected area are shown, after immunostaining for ΔLNGFR (red), GFP (green) and TO-PRO3 staining for nuclear DNA (blue). Fluorescent signals were sequentially acquired from single optical sections and are shown individually and after merging (merge). Original magnification 200× (scale bar, 120 μm) (**f**) Representative cryostatic section from the gut of a ΔLNGFR/GFP MA1 LV transgenic F1 mouse carrying two vector genomes integrated into the germ line and analyzed as in **e**. Original magnification 630× (scale bar, 120 μm). (**g**,**h**) Metabolic correction and robust cell marking of PBL from MLD patients by bidirectional LVs. PBL obtained from MLD patients and normal donors were transduced with matched doses of the indicated bidirectional MA1 LV expressing ARSA/GFP or monocistronic vector expressing either ARSA or GFP, and analyzed 6 d after transduction. (**g**) Dot plot FACS analysis of GFP expression (FL1, *x*-axis; FL2, *y*-axis); percentages and MFI of GFP+ cells are indicated. (**h**) Histogram showing ARSA activity of MLD patient cells transduced by the indicated vectors and of normal donor lymphocytes, assayed by p-nitrocatecholsulphate (PNC) catabolism. Mean ± s.d. ($n = 4$) and significance are shown.

We then assessed the performance of the bidirectional MA1 LVs in more relevant targets for gene therapy applications and by different delivery strategies. We transduced human cord blood hematopoietic progenitor cells (HPCs) and adult donor peripheral blood lymphocytes (PBLs) with ΔLNGFR/GFP MA1 LV *ex vivo*, according to previously optimized protocols[17,18] (**Fig. 4a–d**). Both gene products were coordinately expressed to high levels in a large fraction of HPCs, scored both as immature cells grown in the presence of early acting cytokines (**Fig. 4a**) and after differentiation in liquid culture (**Fig. 4b**) or by clonogenic assay (data not shown). Similarly, we obtained coordinate ΔLNGFR and GFP expression in PBLs transduced in standard conditions of proliferation triggered by CD3/CD28 costimulation (**Fig. 4c**), and as nonproliferating cells, treated only with interleukin (IL)-7 to maintain naive cell properties (**Fig. 4d**). We also performed transplantation studies with transduced murine HPCs and showed stable coordinate expression of ΔLNGFR and GFP in the white blood cells of long-term engrafted mice (**Supplementary Fig. 3** online). Overall, these results validated the new LVs for proficient dual gene transfer in primitive, committed and differentiated hematopoietic cells.

We then injected concentrated ΔLNGFR/GFP MA1 LV in the striata of adult mice and scored transgene expression 4 weeks after injection by confocal microscopy of brain sections immunostained for ΔLNGFR and GFP (**Fig. 4e**). We observed robust coexpression of both transgenes in the brain tissue surrounding the injection site. We also evaluated whether the new bidirectional LVs generated dual-transgenic mouse lines[12]. We obtained transgenic mice at high frequency, as assessed by the presence of vector DNA (more than 50% of newborns), and obtained vector integration in the germ line by crossing some founder mice and analyzing their progeny for vector DNA content and transgene expression. In the two F1 mice analyzed, carrying two and five vector copies in the genome, we found remarkably consistent expression of both transgenes in virtually every cell in the tissues studied, which included brain, liver, spleen, gut, heart, skeletal muscle and kidney (**Fig. 4f** and **Supplementary Fig. 4** online). Vector expression was also easily detectable in the bone marrow and peripheral blood of the same mice, although in less than 100% of the cells, and more clearly for ΔLNGFR than GFP (data not shown). These data indicated that bidirectional LV transgenesis is a rapid and efficient method to obtain robust, stable and coordinate expression of two transgenes in genetically engineered mice. In addition, the data show that the minCMV-PGK bidirectional promoter that we developed governs dual transgene expression in the majority of differentiated tissues of the mouse and maintains this expression after inheritance through the germ line.

Finally, we tested the performance of the MA1 bidirectional LV in a human gene therapy application using PBLs obtained from metachromatic leukodystrophy (MLD) patients, who lack functional arylsulfatase A (ARSA) in the lysosomes. We previously demonstrated that ARSA overexpression to supranormal levels in transplanted hematopoietic stem cells plays a crucial role in preventing disease manifestation in the MLD mouse model, whereas wild-type hematopoietic stem cell transplantation does not[19]. Here we evaluated the possibility of reproducing such conditions of overexpression in patient hematopoietic cells (**Fig. 4g,h**). Both bidirectional and monocistronic ARSA LVs restored enzymatic activity in patient PBLs; ARSA LVs were also overexpressed, as indicated by a two-fold increase in ARSA activity of transduced cells with respect to normal controls, at a transduction efficiency of around 20%. Moreover, using bidirectional LVs allowed genetic correction to be coupled to cell marking with the same efficiencies as those obtained by using similar doses of monocistronic vectors expressing either ARSA or GFP.

These results demonstrate that these bidirectional LVs reach efficient dual-gene transfer and coordinate expression *ex vivo* and *in vivo* and that they outperform currently available technologies. All IRES-based LVs tested displayed some limitations because of the lower efficiency of IRES-dependent gene expression as compared to ^{mRNA}Cap-dependent expression, and to the marked variability in IRES performance according to the type of transgene and target cell tested, as it was also reported after IRES incorporation in other types of gene transfer vectors[6–10]. 2A peptide-based LVs were less efficient in expressing both gene products than were bidirectional vectors and expressed a fraction of unprocessed polyproteins that may display altered stability and novel antigenic features. Moreover, the cellular trafficking of the downstream fusion partner appeared to be dictated by that of the upstream partner, a surprising finding that was also reported in another recent study[20]. The possible consequences of these expression features must be considered before adopting self-processing polyproteins for dual gene therapy.

In summary, when using bicistronic vectors, it will probably be necessary to transfer multiple vector copies or select transduced cells for efficient downstream gene expression to obtain a population expressing two transgenes in the majority of cells. Although selection protocols are compatible with some *ex vivo* gene transfer applications, they may adversely affect the biological properties of gene-corrected cells, in particular when selectable marker expression is inefficient. Even more important, the relatively low efficiency of dual gene expression by bicistronic vectors seriously limits their application to direct *in vivo* gene transfer.

By monitoring transduced cells carrying a single vector copy, we proved that divergent transcription occurred from a single bidirectional promoter, that expression of both transgenes was functionally linked and coordinately regulated, and that bidirectional promoters were consistently active in all types of target cells tested without being silenced or randomly fixed in one direction of transcription, even after cellular differentiation. Although we did not map how close the two opposite core promoters must be for operational linkage, we may expect that close juxtaposition of the fused minimal core promoter to some of the upstream elements in the efficient promoter, as observed in natural promoters between core and upstream elements, may be required. Both the PGK and UBI-C promoters tested in this work drove divergent transcription when fused to a minimal core promoter in the opposite orientation.

Intriguingly, both of these promoters were shown to be intrinsically capable of promoting divergent transcription, although to a lower efficiency on the upstream than the downstream side, when incorporated into the bidirectional expression cassette that we developed. This surprising observation may indicate a specific feature of a class of ubiquitously expressed housekeeping promoters, possibly related to their content of CpG islands (see below and refs. 21–23). Recent studies have also proposed that divergent transcription occurring from a transgenic PGK promoter may explain some unexpected experimental findings[24,25], providing an independent confirmation of our data in a transgenic setting.

We should also note that both promoter placement between two efficient expression cassettes endowed with post-transcriptional regulatory elements enhancing translation, and LV-mediated integration, which has been shown to preferentially target transcribed genes in the chromatin, may contribute to the unraveling of latent transcriptional activity. Although the intrinsic bidirectional activity of the housekeeping promoters tested may not be efficient enough

for exploitation *per se*, without the upstream assembly of core promoter elements described in this work, it provides the basis for the coordinate regulation of dual-gene expression obtained by our new vectors. On the other hand, the propensity of these promoters to drive divergent transcription should be kept in mind when engineering vectors and analyzing transduced cells or tissues[24,25], and may provide a possible mechanism for the frequently observed interference between nearby promoters in the same vector construct.

We have also extended our study to show that the bidirectional design can be successfully applied to a synthetic tissue-specific promoter to obtain coordinated dual gene expression selectively in hepatocyte cell lines. As shown for the ubiquitously expressed promoters described above, the ET enhancer/promoter displayed an intrinsic bidirectional activity that was enhanced by the upstream addition of a minCMV promoter. Further studies will clarify how frequently this feature is to be found among endogenous or synthetic promoters of different types. In addition, by combining bidirectional promoters with bicistronic transcripts one could express more than two transgenes within the same cell, although with the limitations described above.

Inducible bidirectional promoters were originally developed in Tet-regulated expression systems, by duplicating a minimal promoter on both sides of a series of Tet operator repeats to obtain exogenously regulated expression of two transgenes[13,26]. A constitutive bidirectional promoter was recently tested for exogenous gene expression in plant biotechnology[27]. Our results provide a description of synthetic bidirectional promoters that exploit the endogenous transcriptional machinery available to most animal cell types to drive robust and constitutive expression of two divergent transcripts.

In nature, few instances of bidirectional promoters had been documented until recently. Interestingly, a recent survey of the human genome indicated an abundance of divergently transcribed gene pairs, whose transcription start sites are separated by less than 1 kb[28,29]. It is likely that many of the promoter elements found between these gene pairs can initiate transcription in both directions, and contain shared elements that regulate both genes[30]. Although the functional implications of the widespread occurrence of bidirectional promoters and of antisense transcription in the human genome[31] are not fully understood, transcription *per se* may contribute to stabilize open chromatin domains and promoter interaction with the transcriptional machinery. Thus, the synthetic bidirectional promoters that we have developed may mimic a well-represented and evolutionarily conserved feature of eukaryotic transcription, providing a structural basis for their robust performance. The new lentiviral vectors built around these bidirectional promoters will likely advance the reach and safety of gene therapy, as well as increase the power of gene-function, target validation, and animal transgenesis applications.

METHODS

Plasmid construction. All transfer vectors were built from plasmid pCCL.sin.cPPT.PGK.GFP.WPRE[11] using the following previously described sequence elements: EMCV IRESs with the downstream gene coding sequence starting at the 11th ATG of the IRES (wt) the MPMV CTE[14], a minimal CMV core promoter[13], a 1,226 bp fragment from the ubiquitin-C promoter[12]. The ET promoter was generated by fusing a synthetic hepatocyte-specific enhancer—assembled by random ligation of synthetic oligonucleotides coding for binding sites of the following hepatic transcription factors: DPB, C/EBP, HNF1, HNF3, HNF4 and HNF6, and selection for the highest expressing combination (sequence deposited in GenBank with the accession number AY661265)—to the murine transthyretin promoter (M19523: 100–109 bp and M19524:128–350 bp). Detailed information on the generation and experimental validation of the ET promoter will be reported elsewhere (A.D. Simmons, personal communication). The ΔLNGFR-2A-GFP polyprotein was constructed by in-frame insertion of the FMDV 2A peptide sequence between the coding sequences of ΔLNGFR and GFP and cloning the product into pCCL.sin.cPPT.PGK...WPRE vector. Detailed cloning procedures and sequence information are available on request.

Vector production and titration. VSV-pseudotyped third-generation LV were produced by transient four-plasmid cotransfection into 293T cells and purified by ultracentrifugation as described[11], with the modification that 1 μM sodium butyrate was added to the cultures for vector collection. Expression titer of GFP or ΔLNGFR vectors were estimated on HeLa cells by limiting dilution. Vector particle was measured by HIV-1 gag p24 antigen immunocapture (NEN Life Science Products). Vector infectivity was calculated as the ratio between titer and particle for the vector expressing GFP or ΔLNGFR. Vector expression titer in the 293T supernatant ranged from 0.7–1 × 10^7 transducing units^{HeLa}(TU)/ml for monocistronic CMV or PGK vector, from 3–8 × 10^6 TU/ml for bicistronic vectors and bidirectional vectors. Vector infectivity ranged from 0.5–1 × 10^5 TU/ng of p24 for monocistronic CMV or PGK vector, and from 2–6 × 10^4 TU/ng of p24 for bicistronic and bidirectional vectors.

Cell cultures. Continuous cultures of HeLa and 293T cells were maintained in Iscove's modified Dulbecco's medium (IMDM; Sigma) supplemented with 10% fetal bovine serum (FBS; Gibco) and a combination of penicillin-streptomycin and glutamine. Continuous cultures of HepG2, MLP29 and C2C12 cells were maintained in DMEM supplemented as above. To induce myotube differentiation, C2C12 cultures were shifted to 2% FBS. Primary cultures of human umbilical vein endothelial cells (HUVECs), peripheral blood lymphocytes and cord blood $CD34^+$ progenitors were obtained according to protocols approved by the San Raffaele Ethical Committee and maintained as described[11]. $CD34^+$ progenitors were transduced with 5 × 10^7 TU/ml of LV and cultured for at least 7 d in the presence of recombinant human interleukin 6 (rhIL6, 20 ng/ml), recombinant human stem cell factor (rhSCF, 100 ng/ml), recombinant human FLT-3 ligand (rhFLT-3 ligand, 100 ng/ml), all from PeproTech and recombinant human thrombopoietin (rhTPO, 20 ng/ml; Amgen) as described[17]. For differentiating conditions, transduced progenitors were cultured for 10 d in the presence of rhSCF, 50 ng/ml, recombinant human granulocyte monocyte-colony stimulating factor (rhGM-CSF, 20 ng/ml), recombinant human monocyte-colony stimulating factor (rhG-CSF, 20 ng/ml), all from PeproTech. For clonogenic assays, transduced cells were plated at a density of 800 cells/ml in human complete MethoCult medium (StemCell Technologies) and were scored by light and fluorescence microscopy 14 d later.

Human PBLs were purified by Ficoll gradient from healthy blood donors or MLD patients, plated in IMDM 5% human serum, stimulated either with 30 ng/ml anti-CD3 antibodies (Orthoclone) plus 1 μg/ml anti-CD28 antibodies (PharMingen) for 36–48 h, or with 5 ng/ml IL-7 (Boehringer Mannheim-Roche) for 4 d as described[18], and transduced with 0.5–5 × 10^7 TU/ml or 75–125 ng p24/ml of vector for 12 h. After transduction, PBLs were washed and grown in the presence of 6U/ml IL2.

Purification of lineage marker-negative cells from C57BL/6 mouse bone marrow with a magnetic cell depletion technique (StemCell Technologies), *ex vivo* transduction in serum-free StemSpan medium (StemCell Technologies) with 0.5–2 × 10^7 TU/ml of vector and transplantation into lethally irradiated syngenic recipients were done as described[32].

DNA analysis: Southern and real-time PCR. Vector copies per genome were quantified by real-time PCR from 300 ng template DNA extracted from cells by a commercial kit (Qiagen), using one set of primers and probe to detect the LV backbone: LV forward primer, 5′-TGAAAGCGAAAGGGAAACCA-3′; LV reverse primer, 5′-CCGTGCGCGCTTCAG-3′; LV probe, 5′-(VIC)-CTCTCTCG ACGCAGGACT-(TAMRA)-3′. Reactions were carried out according to manufacturer's instructions and analyzed using the ABI Prism 7700 sequence detection system (PE-Applied Biosystem). For Southern blot analysis, DNA was extracted from transduced cells, digested with AfI-II to release the expression cassette from integrated vector DNA and analyzed with a WPRE

probe to detect vector sequences. The average number of integrated vector copies was determined relative to a plasmid standard curve.

These numbers were used to calculate vector integration titer and normalize vector stocks for all subsequent transduction experiments to ensure similar levels of integration for each vector tested.

Mice. CD1, C57BL/6 and FVB mice were purchased from Charles Rivers Laboratories and maintained in specific-pathogen-free conditions. All animal procedures were performed according to protocols approved by the Hospital San Raffaele Institutional Animal Care and Use Committee.

Stereotactic injection. Nine-week-old C57BL/6 mice were anesthetized with intraperitoneal injection of tribromoethanol 1.25% (SIGMA), positioned in a stereotactic frame (David Kopf Instruments) and the skull exposed by a small incision. Two μl of vector concentrate (2×10^6 TU/μl) was injected by a Hamilton syringe with a 33G blunt tip needle (Hamilton) into the left hemisphere striatum (stereotactic coordinates in mm from bregma: AP = +0.74, ML = −1.9 and DV = −3.5 from skull surface) at a rate of 0.2 μl/min. The needle was left in place for an additional 5 min before slow removal.

Transgenesis. Transgenic mice were generated using LVs as described[12]. Briefly, female FVB mice were superovulated with a combination of pregnant mare serum and human chorionic gonadotropin. On average between 20 and 30 embryos were collected per female and microinjected into the perivitelline space with 10–100 pl of 5×10^7 TU/ml LV stock on the same day. Manipulated embryos were immediately implanted into the oviduct of pseudopregnant CD1 mice. Pups were genotyped for the presence of the GFP sequence by PCR. Positive mice were bred to test germline transmission of the transgene. DNA was extracted from the tail and used to quantify vector copy number by real-time PCR in founder and F1 progeny mice.

Flow cytometry and luciferase assay. Transduced cells were grown for at least 4 d before FACS analysis to reach steady state GFP expression and to rule out pseudotransduction. Before FACS analysis, adherent cells were detached with 0.05% trypsin-EDTA, washed and fixed in PBS containing 1% paraformaldehyde (PAF) and 2% FBS. Cells grown in suspension were washed and resuspended in PBS containing 2 μg/ml propidium iodide (PI) (BD Bioscience PharMingen) and 2% FBS. For immunostaining, 10^5 cells were blocked in PBS 5% mouse serum, 5% human serum, 2% FBS for 15 min at 4 °C. After blocking, 10 μl of R-phycoerythrin (RPE)-conjugated antibodies (anti-CD34 and anti-CD13, Dako and anti-ΔLNGFR, BD Bioscience PharMingen) were added and the cells were incubated for 30 min at 4 °C, washed, stained with PI, and analyzed by three-color flow cytometry. Only viable, PI-negative cells were used for the analysis. At least 10,000 events per sample were analyzed. Luciferase was assayed in cell lysates prepared as described by the manufacturer (luciferase assay system, Promega). Relative light units were measured with a Lumat LB9507 luminometer (Berthold) after mixing cell lysates (normalized for protein content measured by a BCA Protein Assay Reagent kit from Pierce) with Luciferase Substrate (Promega).

Western blot analysis. 10^6 transduced cell were collected in Eppendorf tubes, lysed 15 min on ice in 100 μl 50 mM TRIS, pH = 7.6, 150 mM NaCl buffer (TBS) containing 5mM EDTA, 1% NP-40 and a cocktail of protease and phosphatase inhibitors. The lysate was cleared by centrifugation, quantified for protein content by Bradford's method, added to SDS-containing sample buffer for SDS-PAGE on 10% gel, blotted to nitrocellulose paper for 2 h at 60 V, blocked overnight in TBS—0.1% Tween—5% dry milk, washed, incubated with 1:1,000 dilution of rabbit anti-GFP antibodies (Molecular Probes) for 2 h in TBS-Tween, washed, incubated with 1:5,000 dilution of horseradish peroxidase–conjugated donkey anti-rabbit Ig (Amersham), washed and developed with ECL Western Blotting detection reagent (Amersham).

Confocal microscopy analysis. Anesthetized mice were perfused with 0.9% NaCl followed by 4% PAF in PBS. Tissue samples were collected, equilibrated in 20% sucrose in PBS for 48 h at 4 °C, and embedded in optimal-cutting-temperature compound (OCT) for quick freezing. Thick cryostatic sections (10 μm for transgenic mice and 20 μm for stereotactic injected mice were postfixed in PAF and frozen at −80 °C. Sections were blocked with 5% goat serum (Vector Laboratories) in PBS containing 1% bovine serum albumin (BSA) and 0.1% Triton X-100 (PBS-T), and incubated with rabbit affinity-purified GFP antibody (Molecular Probes) and R-phycoerythrin (RPE)-conjugated ΔLNGFR monoclonal antibody (BD Bioscience PharMingen) for 1 h, washed and stained with AlexaFluor488-conjugated goat anti-rabbit antibody (Molecular Probes) in PBS-T and 1% BSA for 1 h. Cell nuclei were stained with TOPRO-3 after 1 h of RNAse treatment (Molecular Probes). Sections were mounted and analyzed by three-laser confocal microscope (Radiance 2100; BioRad). Fluorescent signals from single optical sections were sequentially acquired and analyzed by PhotoShop 7.0 (Adobe). For immunofluorescence analysis of *in vitro* cultures, cells were grown on poly-L-lysine–coated glass coverslips, washed, fixed in PBS containing 1% PAF and 2% FBS, permeabilized in 0.1% Triton X-100 in PBS-1% BSA for 15 min on ice, blocked and immunostained as described above.

ARSA assay. ARSA activity was determined in transduced PBL 6 d after transduction. Cells were collected and lysed in 0.5 M sodium acetate pH 5 at 4 °C for 2 h. 100 μg of sample proteins were incubated with 100 μl of 10 mM p-nitrocatecholsulphate (PNC) for 90 min at 37 °C. The reaction was stopped by 1 ml of 1M NaOH and the product measured in a spectrophotometer at 515 nm. ARSA activity was expressed in nmoles PNC/mg protein/h.

Statistical analysis. Unpaired student's *t* test was performed for statistical evaluation of the data.

Note: Supplementary information is available on the Nature Biotechnology website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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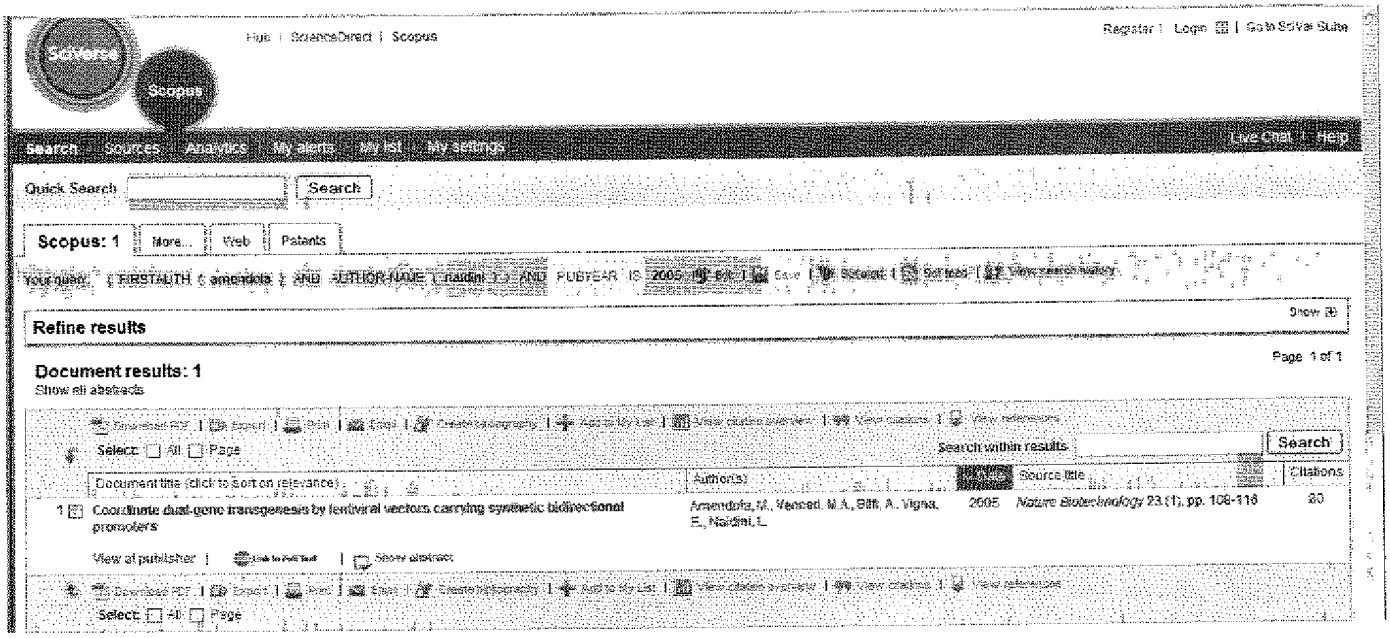
1. Kay, M.A., Glorioso, J.C. & Naldini, L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat. Med.* **7**, 33–40 (2001).
2. Klump, H. *et al.* Retroviral vector-mediated expression of HoxB4 in hematopoietic cells using a novel coexpression strategy. *Gene Ther.* **8**, 811–817 (2001).
3. Emerman, M. & Temin, H.M. Quantitative analysis of gene suppression in integrated retrovirus vectors. *Mol. Cell Biol.* **6**, 792–800 (1986).
4. Miller, A.D. in *Retroviruses* (eds. Coffin, J., Hughes, S.H. & Varmus, H.E.) 437–474 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2000) 10.
5. Furler, S., Paterna, J.C., Weibel, M. & Bueler, H. Recombinant AAV vectors containing the foot and mouth disease virus 2A sequence confer efficient bicistronic gene expression in cultured cells and rat substantia nigra neurons. *Gene Ther.* **8**, 864–873 (2001).
6. Martinez-Salas, E. Internal ribosome entry site biology and its use in expression vectors. *Curr. Opin. Biotechnol.* **10**, 458–464 (1999).
7. Sokolic, R.A. *et al.* A bicistronic retrovirus vector containing a picornavirus internal ribosome entry site allows for correction of X-linked CGD by selection for MDR1 expression. *Blood* **87**, 42–50 (1996).
8. Mizuguchi, H., Xu, Z., Ishii-Watabe, A., Uchida, E. & Hayakawa, T. IRES-dependent second gene expression is significantly lower than cap-dependent first gene expression in a bicistronic vector. *Mol. Ther.* **1**, 376–382 (2000).
9. Hennecke, M. *et al.* Composition and arrangement of genes define the strength of IRES-driven translation in bicistronic mRNAs. *Nucleic Acids Res.* **29**, 3327–3334 (2001).
10. Kozak, M. Alternative ways to think about mRNA sequences and proteins that appear to promote internal initiation of translation. *Gene* **318**, 1–23 (2003).
11. Follenzi, A., Ailles, L.E., Bakovic, S., Geuna, M. & Naldini, L. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. *Nat. Genet.* **25**, 217–222 (2000).
12. Lois, C., Hong, E.J., Pease, S., Brown, E.J. & Baltimore, D. Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. *Science* **295**, 868–872 (2002).

13. Baron, U., Freundlieb, S., Gossen, M. & Bujard, H. Co-regulation of two gene activities by tetracycline via a bidirectional promoter. *Nucleic Acids Res.* **23**, 3605–3606 (1995).
14. Bray, M. *et al.* A small element from the Mason-Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication Rev-independent. *Proc. Natl. Acad. Sci. USA* **91**, 1256–1260 (1994).
15. Qiao, J., Roy, V., Girard, M.H. & Caruso, M. High translation efficiency is mediated by the encephalomyocarditis virus internal ribosomal entry sites if the natural sequence surrounding the eleventh AUG is retained. *Hum. Gene Ther.* **13**, 881–887 (2002).
16. Szymczak, A.L. *et al.* Correction of multi-gene deficiency *in vivo* using a single 'self-cleaving' 2A peptide-based retroviral vector. *Nat. Biotechnol.* **22**, 589–594 (2004).
17. Ailles, L. *et al.* Molecular evidence of lentiviral vector-mediated gene transfer into human self-renewing, multi-potent, long-term NOD/SCID repopulating hematopoietic cells. *Mol. Ther.* **6**, 615–626 (2002).
18. Cavalieri, S. *et al.* Human T lymphocytes transduced by lentiviral vectors in the absence of TCR activation maintain an intact immune competence. *Blood* **102**, 497–505 (2003).
19. Biffi, A. *et al.* Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. *J. Clin. Invest.* **113**, 1118–1129 (2004).
20. de Felipe, P. & Ryan, M.D. Targeting of proteins derived from self-processing polyproteins containing multiple signal sequences. *Traffic* **5**, 616–626 (2004).
21. Gardiner-Garden, M. & Frommer, M. CpG islands in vertebrate genomes. *J. Mol. Biol.* **196**, 261–282 (1987).
22. Lavia, P., Macleod, D. & Bird, A. Coincident start sites for divergent transcripts at a randomly selected CpG-rich island of mouse. *EMBO J.* **6**, 2773–2779 (1987).
23. Johnson, P. & Friedmann, T. Limited bidirectional activity of two housekeeping gene promoters: human HPRT and PGK. *Gene* **88**, 207–213 (1990).
24. Scacheri, P.C. *et al.* Bidirectional transcriptional activity of PGK-neomycin and unexpected embryonic lethality in heterozygote chimeric knockout mice. *Genesis* **30**, 259–263 (2001).
25. Taveau, M. *et al.* Bidirectional transcriptional activity of the Pgk1 promoter and transmission ratio distortion in Capn3-deficient mice. *Genomics* **84**, 592–595 (2004).
26. Unsinger, J., Kroger, A., Hauser, H. & Wirth, D. Retroviral vectors for the transduction of autoregulated, bidirectional expression cassettes. *Mol. Ther.* **4**, 484–489 (2001).
27. Xie, M., He, Y. & Gan, S. Bidirectionalization of polar promoters in plants. *Nat. Biotechnol.* **19**, 677–679 (2001).
28. Trinklein, N.D. *et al.* An abundance of bidirectional promoters in the human genome. *Genome Res.* **14**, 62–66 (2004).
29. Takai, D. & Jones, P.A. Origins of bidirectional promoters: computational analyses of intergenic distance in the human genome. *Mol. Biol. Evol.* **21**, 463–467 (2004).
30. Adachi, N. & Lieber, M.R. Bidirectional gene organization: a common architectural feature of the human genome. *Cell* **109**, 807–809 (2002).
31. Cawley, S. *et al.* Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell* **116**, 499–509 (2004).
32. De Palma, M., Venneri, M.A., Roca, C. & Naldini, L. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat. Med.* **9**, 789–795 (2003).

Exhibit B



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EXPORT DATE: 7 October 2010

Kamata, M., Liang, M., Liu, S., Nagaoka, Y., Chen, I.S.Y.
Live cell monitoring of hiPSC generation and differentiation using differential expression of endogenous microRNAs
(2010) PLoS ONE, 5 (7), pp. 1-12.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77955603296&partnerID=40&md5=524dcc35ce97054425541f7aa11c7d9e
DOCUMENT TYPE: Article
SOURCE: Scopus

Fagoonee, S., Hobbs, R.M., De Chiara, L., Cantarella, D., Piro, R.M., Tolosano, E., Medico, E., Provero, P., Pandolfi, P.P., Silengo, L., Altruda, F.
Generation of functional hepatocytes from mouse germ line cell-derived pluripotent stem cells in vitro
(2010) Stem Cells and Development, 19 (8), pp. 1183-1194. Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77955440543&partnerID=40&md5=ac52c0995e5885a3df5e08a3e8b4b1b9
DOCUMENT TYPE: Article
SOURCE: Scopus

Costa, B., Dettori, D., Lorenzato, A., Bardella, C., Coltella, N., Martino, C., Cammarata, C., Carmeliet, P., Olivero, M., Di Renzo, M.F.
Fumarase tumor suppressor gene and MET oncogene cooperate in upholding transformation and tumorigenesis
(2010) FASEB Journal, 24 (8), pp. 2680-2688.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77955815630&partnerID=40&md5=5d81217ca2a094cbef68d38eeb4e20f7
DOCUMENT TYPE: Article
SOURCE: Scopus

Golder, M., Pruss, C.M., Hegadorn, C., Mewburn, J., Laverty, K., Sponagle, K., Lillicrap, D.

Mutation-specific hemostatic variability in mice expressing common type 2B von Willebrand disease substitutions
(2010) Blood, 115 (23), pp. 4862-4869.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77954693471&partnerID=40&md5=612b4ca8b3bba7aeb1fa03c27b2bc857
DOCUMENT TYPE: Article
SOURCE: Scopus

Na, M., Fan, X.
Design of Ad5F35 vectors for coordinated dual gene expression in candidate human hematopoietic stem cells
(2010) Experimental Hematology, 38 (6), pp. 446-452.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77952953570&partnerID=40&md5=3f548370f458edd2aa7f294a24837802
DOCUMENT TYPE: Article
SOURCE: Scopus

Piersanti, S., Remoli, C., Saggio, I., Funari, A., Michienzi, S., Sacchetti, B., Robey, P.G., Riminucci, M., Bianco, P.
Transfer, analysis, and reversion of the fibrous dysplasia cellular phenotype in human skeletal progenitors
(2010) Journal of Bone and Mineral Research, 25 (5), pp. 1103-1116.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77953532926&partnerID=40&md5=297f024b2bfacc32b3244a5763871a00
DOCUMENT TYPE: Article
SOURCE: Scopus

Kielland, A., Carlsen, H.
Molecular imaging of transcriptional regulation during inflammation
(2010) Journal of Inflammation, 7, art. no. 20, .
http://www.scopus.com/inward/record.url?eid=2-s2.0-77951245418&partnerID=40&md5=a7fe1f129707394b4d72e972be3be6a1
DOCUMENT TYPE: Review
SOURCE: Scopus

Mitchell, J., Paul, P., Chen, H.-J., Morris, A., Payling, M., Falchi, M., Habgood, J., Panoutsou, S., Winkler, S., Tisato, V., Hajitou, A., Smith, B., Vance, C., Shaw, C., Mazarakis, N.D., De Belleroche, J.
Familial amyotrophic lateral sclerosis is associated with a mutation in D-amino acid oxidase
(2010) Proceedings of the National Academy of Sciences of the United States of America, 107 (16), pp. 7556-7561. Cited 2 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77952194773&partnerID=40&md5=43313cb9c4d23c0b10cb05f91df7bd1c
DOCUMENT TYPE: Article
SOURCE: Scopus

Yang, C., Ji, L., Shi, S.S., Yue, W., He, L.J., Nan, X., Pei, X.T.
Differentiation of CD34+ cells into erythroid cells by human fetal liver stromal cells expressing erythropoietin
(2010) Progress in Biochemistry and Biophysics, 37 (4), pp. 381-388.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77954796265&partnerID=40&md5=feee67625029ec5607309b49c8f77de1
DOCUMENT TYPE: Article
SOURCE: Scopus

Lattanzi, A., Neri, M., Maderna, C., di Girolamo, I., Martino, S., Orlacchio, A., Amendola, M., Naldini, L., Gritti, A.
Widespread enzymatic correction of CNS tissues by a single intracerebral injection of therapeutic lentiviral vector in leukodystrophy mouse models
(2010) Human Molecular Genetics, 19 (11), art. no. ddq099, pp. 2208-2227. Cited 2 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77953522837&partnerID=40&md5=88532c893454b3eb46f6c58ca2eef52e
DOCUMENT TYPE: Article
SOURCE: Scopus

Andrianaki, A., Siapati, E.K., Hirata, R.K., Russell, D.W., Vassilopoulos, G.
Dual transgene expression by foamy virus vectors carrying an endogenous bidirectional promoter
(2010) Gene Therapy, 17 (3), pp. 380-388.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77949424086&partnerID=40&md5=77cffda0023779ced1a1dca694010536
DOCUMENT TYPE: Article
SOURCE: Scopus

Maetzig, T., Galla, M., Brugman, M.H., Loew, R., Baum, C., Schambach, A.
Mechanisms controlling titer and expression of bidirectional lentiviral and gammaretroviral vectors
(2010) Gene Therapy, 17 (3), pp. 400-411. Cited 2 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77949424881&partnerID=40&md5=3d8413d9ceca3f8996125e03a9f71355
DOCUMENT TYPE: Article
SOURCE: Scopus

Mann, C.J., Ayuso, E., Anguela, X.M., Bosch, F.
Skeletal muscle metabolism in the pathology and treatment of type 1 diabetes
(2010) Current Pharmaceutical Design, 16 (8), pp. 1002-1020. Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77950270414&partnerID=40&md5=aec9aa5be2b69e3b1db902c754dba7f4
DOCUMENT TYPE: Review
SOURCE: Scopus

Rato, S., Maia, S., Brito, P.M., Resende, L., Pereira, C.F., Moita, C., Freitas, R.P., Moniz-Pereira, J., Hacohen, N., Moita, L.F., Goncalves, J.
Novel HIV-1 knockdown targets identified by an enriched kinases/phosphatases shRNA library using a long-term iterative screen in jurkat T-cells
(2010) PLoS ONE, 5 (2), art. no. e9276, . Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77949530684&partnerID=40&md5=e6c53f116a0930ff5734760886ede86e
DOCUMENT TYPE: Article
SOURCE: Scopus

Tammana, S., Huang, X., Wong, M., Milone, M.C., Ma, L., Levine, B.L., June, C.H., Wagner, J.E., Blazar, B.R., Zhou, X.
4-1BB and CD28 signaling plays a synergistic role in redirecting umbilical cord blood t cells against b-cell malignancies
(2010) Human Gene Therapy, 21 (1), pp. 75-86. Cited 2 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-74949124101&partnerID=40&md5=46ab864a5c72dbb80aa382bfffbc13b1
DOCUMENT TYPE: Article
SOURCE: Scopus

Audisio, C., Raimondo, S., Nicolino, S., Gambarotta, G., Di Scipio, F., Macrì, L., Montarolo, F., Giacobini-Robecchi, M.G., Porporato, P., Filigheddu, N., Graziani, A., Geuna, S., Perroteau, I.
Morphological and biomolecular characterization of the neonatal olfactory bulb ensheathing cell line
(2009) Journal of Neuroscience Methods, 185 (1), pp. 89-98.
http://www.scopus.com/inward/record.url?eid=2-s2.0-70350732786&partnerID=40&md5=2bc3eb042d0ca7d7b7c91a768ed02ccf
DOCUMENT TYPE: Article
SOURCE: Scopus

Bobisse, S., Rondina, M., Merlo, A., Tisato, V., Mandruzzato, S., Amendola, M., Naldini, L., Willemsen, R.A., Debets, R., Zanovello, P., Rosato, A.
Reprogramming T lymphocytes for melanoma adoptive immunotherapy by T-cell receptor gene transfer with lentiviral vectors
(2009) Cancer Research, 69 (24), pp. 9385-9394. Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-73649118221&partnerID=40&md5=a998ca61da4084065a3ee09e0aab6869
DOCUMENT TYPE: Article
SOURCE: Scopus

Circosta, P., Granziero, L., Follenzi, A., Vigna, E., Stella, S., Vallario, A., Elia, A.R., Gammaitoni, L., Vitaggio, K., Orso, F., Geuna, M., Sangiolo, D., Todorovic, M., Giachino, C., Cignetti, A.
T cell receptor (TCR) gene transfer with lentiviral vectors allows efficient redirection of tumor specificity in naive and memory T cells without prior stimulation of endogenous TCR
(2009) Human Gene Therapy, 20 (12), pp. 1576-1588. Cited 3 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-70349929446&partnerID=40&md5=5ced50d05c77faa17c915effe1060b16
DOCUMENT TYPE: Article
SOURCE: Scopus

Pluta, K., Kacprzak, M.M.
Use of HIV as a gene transfer vector
(2009) Acta Biochimica Polonica, 56 (4), pp. 531-595. Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-77449118071&partnerID=40&md5=710ea2cee2c11c192773454f6b2ee7c
DOCUMENT TYPE: Review
SOURCE: Scopus

Howorth, P.W., Thornton, S.R., O'Brien, V., Smith, W.D., Nikiforova, N., Teschemacher, A.G., Pickering, A.E.
Retrograde viral vector-mediated inhibition of pontospinal noradrenergic neurons causes hyperalgesia in rats
(2009) Journal of Neuroscience, 29 (41), pp. 12855-12864.
http://www.scopus.com/inward/record.url?eid=2-s2.0-70350455045&partnerID=40&md5=521a3adf959e60e61d8d5ecaac1fa85d
DOCUMENT TYPE: Article
SOURCE: Scopus

Ji, L., Liu, Y.-X., Yang, C., Yue, W., Shi, S.-S., Bai, C.-X., Xi, J.-F., Nan, X., Pei, X.-T.
Self-renewal and pluripotency is maintained in human embryonic stem cells by co-culture with human fetal liver stromal cells expressing hypoxia inducible factor 1α
(2009) Journal of Cellular Physiology, 221 (1), pp. 54-66. Cited 3 times.

http://www.scopus.com/inward/record.url?eid=2-s2.0-69249225654&partnerID=40&md5=185cd1f0bcc4507029acdcfe7e491152
DOCUMENT TYPE: Article
SOURCE: Scopus

Neagu, M.R., Ziegler, P., Pertel, T., Strambio-De-Castillia, C., Grütter, C., Martinetti, G., Mazzucchelli, L., Grütter, M., Manz, M.G., Luban, J.
Potent inhibition of HIV-1 by TRIM5-cyclophilin fusion proteins engineered from human components
(2009) Journal of Clinical Investigation, 119 (10), pp. 3035-3047. Cited 6 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-70349655695&partnerID=40&md5=f286a7648ab151a1cebe56f9d9701dd3
DOCUMENT TYPE: Article
SOURCE: Scopus

Salerno, E., Scaglione, B.J., Coffman, F.D., Brown, B.D., Baccarini, A., Fernandes, H., Marti, G., Raveche, E.S.
Correcting miR-15a/16 genetic defect in New Zealand Black mouse model of CLL enhances drug sensitivity
(2009) Molecular Cancer Therapeutics, 8 (9), pp. 2684-2692. Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-70349495864&partnerID=40&md5=4376b2c62692b1d771f57fef8117a119
DOCUMENT TYPE: Article
SOURCE: Scopus

Crome, S.Q., Wang, A.Y., Kang, C.Y., Levings, M.K.
The role of retinoic acid-related orphan receptor variant 2 and IL-17 in the development and function of human CD4+ T cells
(2009) European Journal of Immunology, 39 (6), pp. 1480-1493. Cited 6 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-67649525188&partnerID=40&md5=00b44a353e0aeb18a608ec087a0d71fa
DOCUMENT TYPE: Article
SOURCE: Scopus

Ibrahimi, A., Velde, G.V., Reumers, V., Toelen, J., Thiry, I., Vandeputte, C., Vets, S., Deroose, C., Bormans, G., Baekelandt, V., Debyser, Z., Gijsbers, R.
Highly efficient multicistronic lentiviral vectors with peptide 2A sequences
(2009) Human Gene Therapy, 20 (8), pp. 845-860. Cited 4 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-68949216338&partnerID=40&md5=169c93532a2630c19b7392bcc0e16b64
DOCUMENT TYPE: Article
SOURCE: Scopus

Jones, S., Peng, P.D., Yang, S., Hsu, C., Cohen, C.J., Zhao, Y., Abad, J., Zheng, Z., Rosenberg, S.A., Morgan, R.A.
Lentiviral vector design for optimal T cell receptor gene expression in the transduction of peripheral blood lymphocytes and tumor-infiltrating lymphocytes
(2009) Human Gene Therapy, 20 (6), pp. 630-640. Cited 7 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-68849130990&partnerID=40&md5=01028b4d950efe5a3f1798c8766682f4
DOCUMENT TYPE: Article
SOURCE: Scopus

Tian, J., Andreadis, S.T.

Independent and high-level dual-gene expression in adult stem-progenitor cells from a single lentiviral vector
(2009) Gene Therapy, 16 (7), pp. 874-884. Cited 7 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-67749086241&partnerID=40&md5=d99e094a2fa89db4e78ab7ce5868e9b9
DOCUMENT TYPE: Article
SOURCE: Scopus

Uckert, W., Schumacher, T.N.M.
TCR transgenes and transgene cassettes for TCR gene therapy: Status in 2008
(2009) Cancer Immunology, Immunotherapy, 58 (5), pp. 809-822. Cited 17 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-61349172434&partnerID=40&md5=4c3a72f3ca379343a19d3d08c32702e4
DOCUMENT TYPE: Review
SOURCE: Scopus

Kutner, R.H., Zhang, X.-Y., Reiser, J.
Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors
(2009) Nature Protocols, 4 (4), pp. 495-505. Cited 10 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-64749107970&partnerID=40&md5=c2ab6b6c0c91836b75b43845ef8d3a9c
DOCUMENT TYPE: Article
SOURCE: Scopus

Bao, L., Guo, H., Huang, X., Tammana, S., Wong, M., McIvor, R.S., Zhou, X.
High-titer lentiviral vectors stimulate fetal calf serum-specific human CD4 T-cell responses: Implications in human gene therapy
(2009) Gene Therapy, 16 (6), pp. 788-795.
http://www.scopus.com/inward/record.url?eid=2-s2.0-67349212906&partnerID=40&md5=4fac091c158be3a17459ed92d964b011
DOCUMENT TYPE: Article
SOURCE: Scopus

Amendola, M., Passerini, L., Pucci, F., Gentner, B., Bacchetta, R., Naldini, L.
Regulated and multiple miRNA and siRNA delivery into primary cells by a lentiviral platform
(2009) Molecular Therapy, 17 (6), pp. 1039-1052. Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-67349250445&partnerID=40&md5=2a67e79b9ede3a5117cc914669e81ec6
DOCUMENT TYPE: Article
SOURCE: Scopus

Pei, H., Yang, Y., Xi, J., Bai, Z., Yue, W., Nan, X., Bai, C., Wang, Y., Pei, X.
Lineage restriction and differentiation of human embryonic stem cells into hepatic progenitors and zone 1 hepatocytes
(2009) Tissue Engineering - Part C: Methods, 15 (1), pp. 95-104. Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-66149153524&partnerID=40&md5=dc809af1fe63c958ea190b5958e52e21
DOCUMENT TYPE: Article
SOURCE: Scopus

Cobbaert, C.M., Baadenhuijsen, H., Weykamp, C.W.
Prime time for enzymatic creatinine methods in pediatrics

(2009) Clinical Chemistry, 55 (3), pp. 549-558. Cited 7 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-62149102286&partnerID=40&md5=20b7b3b8a9e699aa6d387398a6032b50
DOCUMENT TYPE: Article
SOURCE: Scopus

Benzekhroufa, K., Liu, B.-H., Teschemacher, A.G., Kasparov, S.
Targeting central serotonergic neurons with lentiviral vectors based on a transcriptional amplification strategy
(2009) Gene Therapy, 16 (5), pp. 681-688. Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-67349222113&partnerID=40&md5=5e48a05ff2fb941304b1008c55e09693
DOCUMENT TYPE: Article
SOURCE: Scopus

Valori, C.F., Ning, K., Wyles, M., Azzouz, M.
Development and applications of non-HIV-based lentiviral vectors in neurological disorders
(2008) Current Gene Therapy, 8 (6), pp. 406-418. Cited 7 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-60649100499&partnerID=40&md5=7a9ccb62df4b2985e83ee14cd0b79332
DOCUMENT TYPE: Review
SOURCE: Scopus

Vigna, E., Pacchiana, G., Mazzone, M., Chiriaco, C., Fontani, L., Basilico, C., Pennacchietti, S., Comoglio, P.M.
"Active" cancer immunotherapy by anti-met antibody gene transfer
(2008) Cancer Research, 68 (22), pp. 9176-9183. Cited 4 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-56449108311&partnerID=40&md5=2e1ab206f165d4b34b633b0fab8ad598
DOCUMENT TYPE: Article
SOURCE: Scopus

Chan, J., Kumar, S., Fisk, N.M.
First trimester embryo-fetoscopic and ultrasound-guided fetal blood sampling for ex vivo viral transduction of cultured human fetal mesenchymal stem cells
(2008) Human Reproduction, 23 (11), pp. 2427-2437. Cited 4 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-54149104472&partnerID=40&md5=3d66977daa9ba0fbe3418c7d9ffdeb61
DOCUMENT TYPE: Article
SOURCE: Scopus

Pfützner, W.
Retroviral bicistronic vectors
(2008) Drug News and Perspectives, 21 (9), pp. 473-480. Cited 3 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-61849097214&partnerID=40&md5=3f59f2f44a2785ac93bb4194948972df
DOCUMENT TYPE: Review
SOURCE: Scopus

Wilson, A.A., Kwok, L.W., Hovav, A.-H., Ohle, S.J., Little, F.F., Fine, A., Kotton, D.N.
Sustained expression of α1-antitrypsin after transplantation of manipulated hematopoietic stem cells
(2008) American Journal of Respiratory Cell and Molecular Biology, 39 (2), pp. 133-141. Cited 3 times.

http://www.scopus.com/inward/record.url?eid=2-s2.0-48649084090&partnerID=40&md5=830ddde7bbf6c2e5b365f302cfab8303
DOCUMENT TYPE: Article
SOURCE: Scopus

Yang, S., Cohen, C.J., Peng, P.D., Zhao, Y., Cassard, L., Yu, Z., Zheng, Z., Jones, S., Restifo, N.P., Rosenberg, S.A., Morgan, R.A.
Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition
(2008) Gene Therapy, 15 (21), pp. 1411-1423. Cited 18 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-54549106702&partnerID=40&md5=897ce5553c76703ff5545385f4a85571
DOCUMENT TYPE: Article
SOURCE: Scopus

Gajavelli, S., Castellanos, D.A., Furmanski, O., Schiller, P.C., Sagen, J.
Sustained analgesic peptide secretion and cell labeling using a novel genetic modification
(2008) Cell Transplantation, 17 (4), pp. 445-455. Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-44149115476&partnerID=40&md5=02193ae8fbfb0b619e0c63094c6c73de
DOCUMENT TYPE: Conference Paper
SOURCE: Scopus

Liu, B., Paton, J.F., Kasparov, S.
Viral vectors based on bidirectional cell-specific mammalian promoters and transcriptional amplification strategy for use in vitro and in vivo
(2008) BMC Biotechnology, 8, art. no. 49, . Cited 14 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-44449120235&partnerID=40&md5=df3b4e11af8ec46a98a800812c24956c
DOCUMENT TYPE: Article
SOURCE: Scopus

Nicholls, R.E., Alarcon, J.M., Malleret, G., Carroll, R.C., Grody, M., Vronskaya, S., Kandel, E.R.
Transgenic Mice Lacking NMDAR-Dependent LTD Exhibit Deficits in Behavioral Flexibility
(2008) Neuron, 58 (1), pp. 104-117. Cited 13 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-41549157232&partnerID=40&md5=d5d898b6a9959df9e5bca3eed18dc18e
DOCUMENT TYPE: Article
SOURCE: Scopus

Miretti, S., Roato, I., Taulli, R., Ponzetto, C., Cilli, M., Olivero, M., Di Renzo, M.F., Godio, L., Albini, A., Buracco, P., Ferracini, R.
A mouse model of pulmonary metastasis from spontaneous osteosarcoma monitored in vivo by luciferase imaging
(2008) PLoS ONE, 3 (3), art. no. e1828, . Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-46649087152&partnerID=40&md5=4d122bfd9f7f4ce06c8712c9c55f7381
DOCUMENT TYPE: Article
SOURCE: Scopus

Passerini, L., Allan, S.E., Battaglia, M., Di Nunzio, S., Alstad, A.N., Levings, M.K., Roncarolo, M.G., Bacchetta, R.
STAT5-signaling cytokines regulate the expression of FOXP3 in CD4+CD25+ regulatory T cells and CD4+ CD25- effector T cells

(2008) International Immunology, 20 (3), pp. 421-431. Cited 27 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-40049087686&partnerID=40&md5=965e5e11e3b7a3739e967f7d890cb060
DOCUMENT TYPE: Article
SOURCE: Scopus

Yuan, H., Zhang, P., Qin, L., Chen, L., Shi, S., Lu, Y., Yan, F., Bai, C., Nan, X., Liu, D., Li, Y., Yue, W., Pei, X.
Overexpression of SPINDLIN1 induces cellular senescence, multinucleation and apoptosis
(2008) Gene, 410 (1), pp. 67-74. Cited 4 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-40849083563&partnerID=40&md5=1f3d8cbde034a225693e67f69c47454d
DOCUMENT TYPE: Article
SOURCE: Scopus

Gascón, S., Paez-Gomez, J.A., Díaz-Guerra, M., Scheiffele, P., Scholl, F.G.
Dual-promoter lentiviral vectors for constitutive and regulated gene expression in neurons
(2008) Journal of Neuroscience Methods, 168 (1), pp. 104-112. Cited 10 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-38049000861&partnerID=40&md5=7fa9a5351ba2a4c2882ddfbefcc0a504
DOCUMENT TYPE: Article
SOURCE: Scopus

Allan, S.E., Alstad, A.N., Merindol, N., Crellin, N.K., Amendola, M., Bacchetta, R., Naldini, L., Roncarolo, M.G., Soudeyns, H., Levings, M.K.
Generation of potent and stable human CD4+ T regulatory cells by activation-independent expression of FOXP3
(2008) Molecular Therapy, 16 (1), pp. 194-202. Cited 35 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-37549032709&partnerID=40&md5=0ac1b8123a958784a6d940e01bb0b969
DOCUMENT TYPE: Article
SOURCE: Scopus

Brown, B.D., Cantore, A., Annoni, A., Sergi, L.S., Lombardo, A., Della Valle, P., D'Angelo, A., Naldini, L.
A microRNA-regulated lentiviral vector mediates stable correction of hemophilia B mice
(2007) Blood, 110 (13), pp. 4144-4152. Cited 48 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-36849053006&partnerID=40&md5=fa095ec73f9a473e59b76332e4ed8a1d
DOCUMENT TYPE: Article
SOURCE: Scopus

Rosa, A., Ballarino, M., Sorrentino, A., Sthandier, O., De Angelis, F.G., Marchioni, M., Masella, B., Guarini, A., Fatica, A., Peschle, C., Bozzoni, I.
The interplay between the master transcription factor PU.1 and miR-424 regulates human monocyte/macrophage differentiation
(2007) Proceedings of the National Academy of Sciences of the United States of America, 104 (50), pp. 19849-19854. Cited 43 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-38049146022&partnerID=40&md5=9eb830ee3e47c85864a0b68e2ed31ba8
DOCUMENT TYPE: Article
SOURCE: Scopus

He, Y., Munn, D., Falo Jr., L.D.

Recombinant lentivector as a genetic immunization vehicle for antitumor immunity
(2007) Expert Review of Vaccines, 6 (6), pp. 913-924. Cited 8 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-37149001171&partnerID=40&md5=8986cc41795f3fa6332a8bec9e7a9c5d
DOCUMENT TYPE: Review
SOURCE: Scopus

Wang, S., Yao, Q., Tao, J., Qiao, Y., Zhang, Z.
Co-ordinate expression of glycine betaine synthesis genes linked by the FMDV 2A region in a single open reading frame in Pichia pastoris
(2007) Applied Microbiology and Biotechnology, 77 (4), pp. 891-899. Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-36348979912&partnerID=40&md5=61b73d23ca626b574948b37a08f52bcf
DOCUMENT TYPE: Article
SOURCE: Scopus

Brown, B.D., Gentner, B., Cantore, A., Colleoni, S., Amendola, M., Zingale, A., Baccarini, A., Lazzari, G., Galli, C., Naldini, L.
Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state
(2007) Nature Biotechnology, 25 (12), pp. 1457-1467. Cited 68 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-36849060584&partnerID=40&md5=df7018d935f6d450d37ef723803a4273
DOCUMENT TYPE: Article
SOURCE: Scopus

Collins, P.J., Kobayashi, Y., Nguyen, L., Trinklein, N.D., Myers, R.M.
The ets-related transcription factor GABP directs bidirectional transcription
(2007) PLoS Genetics, 3 (11), pp. 2247-2255.
http://www.scopus.com/inward/record.url?eid=2-s2.0-37349088130&partnerID=40&md5=270c3fc9389290e4c3edce2b2fe8e94e
DOCUMENT TYPE: Article
SOURCE: Scopus

Liu, Q.-B., Li, Y.-H., Yang, Y.-X., Yuan, H.-F., Zhang, R., Qin, L.-P., Lü, Y., Zhang, S.-N., Sun, D.-J., Chen, L., Bai, C.-X., Nan, X., Wang, Y.-F., Pei, X.-T.
NRSF plays a regulatory role in human insulin gene transcription
(2007) Progress in Biochemistry and Biophysics, 34 (9), pp. 952-959. Cited 2 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-37749013483&partnerID=40&md5=c5fb4bb98bf5d15254390216e31843fb
DOCUMENT TYPE: Article
SOURCE: Scopus

Haviernik, P., Zhang, Y., Bunting, K.D.
Retroviral transduction of murine hematopoietic stem cells
(2007) Methods in Molecular Biology, 430, pp. 229-241.
http://www.scopus.com/inward/record.url?eid=2-s2.0-40549136465&partnerID=40&md5=3a9b2309b538ff240e243d550a41a671
DOCUMENT TYPE: Article
SOURCE: Scopus

Schambach, A., Baum, C.

Vector design for expression of O6-methylguanine-DNA methyltransferase in hematopoietic cells
(2007) DNA Repair, 6 (8), pp. 1187-1196. Cited 6 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34447331660&partnerID=40&md5=8459595e32c3f7f0bbde9bed45c17046
DOCUMENT TYPE: Article
SOURCE: Scopus

Hioki, H., Kameda, H., Nakamura, H., Okunomiya, T., Ohira, K., Nakamura, K., Kuroda, M., Furuta, T., Kaneko, T.
Efficient gene transduction of neurons by lentivirus with enhanced neuron-specific promoters
(2007) Gene Therapy, 14 (11), pp. 872-882. Cited 19 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34249067496&partnerID=40&md5=fe4d4fa2fb9188493491c198fdec627d
DOCUMENT TYPE: Article
SOURCE: Scopus

Bobisse, S., Zanovello, P., Rosato, A.
T-cell receptor gene transfer by lentiviral vectors in adoptive cell therapy
(2007) Expert Opinion on Biological Therapy, 7 (6), pp. 893-906. Cited 6 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34250716494&partnerID=40&md5=11da14681c8d1a224d0ff766cd3c6f89
DOCUMENT TYPE: Review
SOURCE: Scopus

Politi, L.S.
MR-based imaging of neural stem cells
(2007) Neuroradiology, 49 (6), pp. 523-534. Cited 16 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34249890215&partnerID=40&md5=15771bd2abbbf56a9a7525acc22737fe
DOCUMENT TYPE: Review
SOURCE: Scopus

Voena, C., Conte, C., Ambrogio, C., Erba, E.B., Boccalatte, F., Mohammed, S., Jensen, O.N., Palestro, G., Inghirami, G., Chiarle, R.
The tyrosine phosphatase Shp2 interacts with NPM-ALK and regulates anaplastic lymphoma cell growth and migration
(2007) Cancer Research, 67 (9), pp. 4278-4286. Cited 18 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34249277428&partnerID=40&md5=e1f01def7c3416b9b993c5298fe4894e
DOCUMENT TYPE: Article
SOURCE: Scopus

Raman, V., Pathak, A.P., Glunde, K., Artemov, D., Bhujwalla, Z.M.
Magnetic resonance imaging and spectroscopy of transgenic models of cancer
(2007) NMR in Biomedicine, 20 (3), pp. 186-199. Cited 9 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34249747982&partnerID=40&md5=17414300c543ae283448dbba73ab9c12
DOCUMENT TYPE: Review
SOURCE: Scopus

Fraldi, A., Biffi, A., Lombardi, A., Visigalli, I., Pepe, S., Settembre, C., Nusco, E., Auricchio, A., Naldini, L., Ballabio, A., Cosma, M.P.
SUMF1 enhances sulfatase activities in vivo in five sulfatase deficiencies
(2007) Biochemical Journal, 403 (2), pp. 305-312. Cited 14 times.

http://www.scopus.com/inward/record.url?eid=2-s2.0-34247137829&partnerID=40&md5=6681547abf92b4b34e875c722aae328d
DOCUMENT TYPE: Article
SOURCE: Scopus

Li, Y., Zhang, R., Qiao, H., Zhang, H., Wang, Y., Yuan, H., Liu, Q., Liu, D., Chen, L., Pei, X.
Generation of insulin-producing cells from PDX-1 gene-modified human mesenchymal stem cells
(2007) Journal of Cellular Physiology, 211 (1), pp. 36-44. Cited 34 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33847644576&partnerID=40&md5=bd252e6f239d9ea957dc860acb1da4c0
DOCUMENT TYPE: Article
SOURCE: Scopus

Filigheddu, N., Gnocchi, V.F., Coscia, M., Cappelli, M., Porporato, P.E., Taulli, R., Traini, S., Baldanzi, G., Chianale, F., Cutrupi, S., Arnoletti, E., Ghè, C., Fubini, A., Surico, N., Sinigaglia, F., Ponzetto, C., Muccioli, G., Crepaldi, T., Graziani, A.
Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells
(2007) Molecular Biology of the Cell, 18 (3), pp. 986-994. Cited 28 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33947186298&partnerID=40&md5=8d3d92e45b1dd4a17118c684c44ded13
DOCUMENT TYPE: Article
SOURCE: Scopus

Crellin, N.K., Garcia, R.V., Levings, M.K.
Altered activation of AKT is required for the suppressive function of human CD4+CD25+ T regulatory cells
(2007) Blood, 109 (5), pp. 2014-2022. Cited 30 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33847397525&partnerID=40&md5=9595bea47c7d3943e9708ebe280f2792
DOCUMENT TYPE: Article
SOURCE: Scopus

Zhou, B.Y., Ye, Z., Chen, G., Gao, Z.P., Zhang, Y.A., Cheng, L.
Inducible and reversible transgene expression in human stem cells after efficient and stable gene transfer
(2007) Stem Cells, 25 (3), pp. 779-789. Cited 26 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33847642244&partnerID=40&md5=9344ddcd0328bb6eef20e9f7de33ce50
DOCUMENT TYPE: Article
SOURCE: Scopus

Copeland, C.S., Mann, V.H., Brindley, P.J.
Both sense and antisense strands of the LTR of the Schistosoma mansoni Pao-like retrotransposon Sinbad drive luciferase expression
(2007) Molecular Genetics and Genomics, 277 (2), pp. 161-170. Cited 3 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33846845073&partnerID=40&md5=a049d83b4810715161a14dbf33497490
DOCUMENT TYPE: Article
SOURCE: Scopus

Xi, J.-F., Wang, Y.-F., Zhang, P., Yuan, H.-F., Shi, S.-S., Yan, F., Chen, L., Bai, C.-X., Nan, X., Pei, X.-T.

Establishment of fetal liver stroma cell lines which stably express basic fibroblast growth factor by lentiviral system
(2007) Progress in Biochemistry and Biophysics, 34 (2), pp. 207-214. Cited 1 time.
http://www.scopus.com/inward/record.url?eid=2-s2.0-34248559840&partnerID=40&md5=340ac61cd9a7b34bd655e11d2c582211
DOCUMENT TYPE: Article
SOURCE: Scopus

Cobrinik, D., Francis, R.O., Abramson, D.H., Lee, T.C.
Rb induces a proliferative arrest and curtails Brn-2 expression in retinoblastoma cells
(2006) Molecular Cancer, 5, art. no. 72, . Cited 5 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33846664769&partnerID=40&md5=1c271aeeac0ffeb7367f83e304d4fb56
DOCUMENT TYPE: Article
SOURCE: Scopus

Harper, S.Q., Staber, P.D., Beck, C.R., Fineberg, S.K., Stein, C., Ochoa, D., Davidson, B.L.
Optimization of feline immunodeficiency virus vectors for RNA interference
(2006) Journal of Virology, 80 (19), pp. 9371-9380. Cited 11 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33748947808&partnerID=40&md5=a121aeb11555b663384de2423477d230
DOCUMENT TYPE: Article
SOURCE: Scopus

Brown, B.D., Venneri, M.A., Zingale, A., Sergi, L.S., Naldini, L.
Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer
(2006) Nature Medicine, 12 (5), pp. 585-591. Cited 99 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33646564701&partnerID=40&md5=db86da15f4481b67c0870b4aa970cf43
DOCUMENT TYPE: Article
SOURCE: Scopus

Chinnasamy, D., Milsom, M.D., Shaffer, J., Neuenfeldt, J., Shaaban, A.F., Margison, G.P., Fairbairn, L.J., Chinnasamy, N.
Multicistronic lentiviral vectors containing the FMDV 2A cleavage factor demonstrate robust expression of encoded genes at limiting MOI
(2006) Virology Journal, 3, art. no. 14, . Cited 24 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-33646561281&partnerID=40&md5=f3549c29252223bc247b2fbab8c5aaf7
DOCUMENT TYPE: Article
SOURCE: Scopus

Jakobsson, J., Lundberg, C.
Lentiviral vectors for use in the central nervous system
(2006) Molecular Therapy, 13 (3), pp. 484-493. Cited 34 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-32944473438&partnerID=40&md5=bcefe76fec9c341f36caad680f85ad7a
DOCUMENT TYPE: Review
SOURCE: Scopus

Barde, I., Zanta-Boussif, M.A., Paisant, S., Leboeuf, M., Rameau, P., Delenda, C., Danos, O.

Efficient control of gene expression in the hematopoietic system using a single Tet-on inducible lentiviral vector
(2006) Molecular Therapy, 13 (2), pp. 382-390. Cited 22 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-30344473326&partnerID=40&md5=3f99ea25dcb6986d42c66caad4678f41
DOCUMENT TYPE: Article
SOURCE: Scopus

De Felipe, P., Luke, G.A., Hughes, L.E., Gani, D., Halpin, C., Ryan, M.D.
E unum pluribus: Multiple proteins from a self-processing polyprotein
(2006) Trends in Biotechnology, 24 (2), pp. 68-75. Cited 27 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-31444448624&partnerID=40&md5=366af33b1e4c4e660e919385ebe8321a
DOCUMENT TYPE: Review
SOURCE: Scopus

Karpova, A.Y., Tervo, D.G.R., Gray, N.W., Svoboda, K.
Rapid and reversible chemical inactivation of synaptic transmission in genetically targeted neurons
(2005) Neuron, 48 (5), pp. 727-735. Cited 44 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-28744446111&partnerID=40&md5=a1265a9770c724b784fb7919040acc4f
DOCUMENT TYPE: Article
SOURCE: Scopus

Sammarco, M.C., Grabczyk, E.
A series of bidirectional tetracycline-inducible promoters provides coordinated protein expression
(2005) Analytical Biochemistry, 346 (2), pp. 210-216. Cited 6 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-27644440016&partnerID=40&md5=37a397774ceb9f74cce294086daa7e28
DOCUMENT TYPE: Article
SOURCE: Scopus

Sinn, P.L., Sauter, S.L., McCray Jr., P.B.
Gene therapy progress and prospects: Development of improved lentiviral and retroviral vectors - Design, biosafety, and production
(2005) Gene Therapy, 12 (14), pp. 1089-1098. Cited 98 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-22744455256&partnerID=40&md5=4490ebce65516be1e53e2f29a8aed155
DOCUMENT TYPE: Review
SOURCE: Scopus

De Palma, M., Montini, E., Santoni De Sio, F.R., Benedicenti, F., Gentile, A., Medico, E., Naldini, L.
Promoter trapping reveals significant differences in integration site selection between MLV and HIV vectors in primary hematopoietic cells
(2005) Blood, 105 (6), pp. 2307-2315. Cited 79 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-15244346492&partnerID=40&md5=f4c094e3b26c92c1c479584b35e951f0
DOCUMENT TYPE: Article
SOURCE: Scopus